REMARKS

With the entry of the present amendment, claims 1-21, 23-27, 29-35 and 44-... are in this application. Claim1 has been amended to incorporate the limitations of claim 43, which has been canceled to avoid redundancy. Claims 2 has been amended to depend from claim 1, rather than from claim 43. Claims 3 and 10 have been amended to better claim the invention. Claim 46 (withdrawn) has been amended to correct an obvious inadvertent typographical error. The amendments to the claims and new claims 49-52 are supported by as-filed Specification and by the as-filed claims. None of the amendments made herein constitutes the addition of new matter.

The Rejections under 35 U.S.C. 103

Claims 1-16, 18-21, 23-24 and 29-30 remain rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Heinrich et al. (2000) PNAS 97:8229-8232 in view of Gossen et al. (2001); Pane et al. (2002) and Fussenegger et al. (1997). Applicants respectfully traverse this rejection.

Heinrich is said to teach a tetracycline repressible female-specific lethal genetic system in Drosophila.

Applicants provide the following discussion to clarify the salient and unique features of the present invention, reiterating distinctions presented in the prior amendment. The key aspect of the invention is a system which employs positive feedback to control gene expression. In any kind of feedback system, an output signal plays into the input. In the present biological system, the expression product of the control factor gene (for instance, tTA, in certain embodiments, could be considered analogous to the output of the first element. Once expressed, tTA induces expression from the second element. This is, at least in part, known from the art, including Heinrich.

However, Applicant respectfully point out that what is new and different in the present claimed invention is that the tTA expression product takes part in its own positive feedback loop. The first element comprises the control factor gene and a promoter for same. Notably, the claim requires that the expression product of the control factor gene of the first element serves as a positive transcriptional control factor for both the at least one first promoter in said first element. That is to say, the tTA expression product acts on its own promoter in the first element so that the more tTA that is expressed, the greater the action on the tTA promoter to drive expression of yet more tTA from the first element. Thus using the sound system analogy, the tTA output is played (or fed) back into the input in the first element by effectively driving its own expression (in the absence of tetracycline). This is the positive feedback loop at the heart of the invention.

At the same time, the expressed tTA in the absence of tetracycline is also driving expression from the second element.

For the purposes of further clarification, the use of figures may be helpful, and this is best explained with reference to certain preferred embodiments, such as in claim 4. In such a system, the first and second elements comprise the tetO enhancer (the claim also specifying that the control factor gene product is the preferred tTA or a variant). Thus, the system of claim 3 would be:

First element:

[tetO] [suitable promoter] – [tTA or variant]

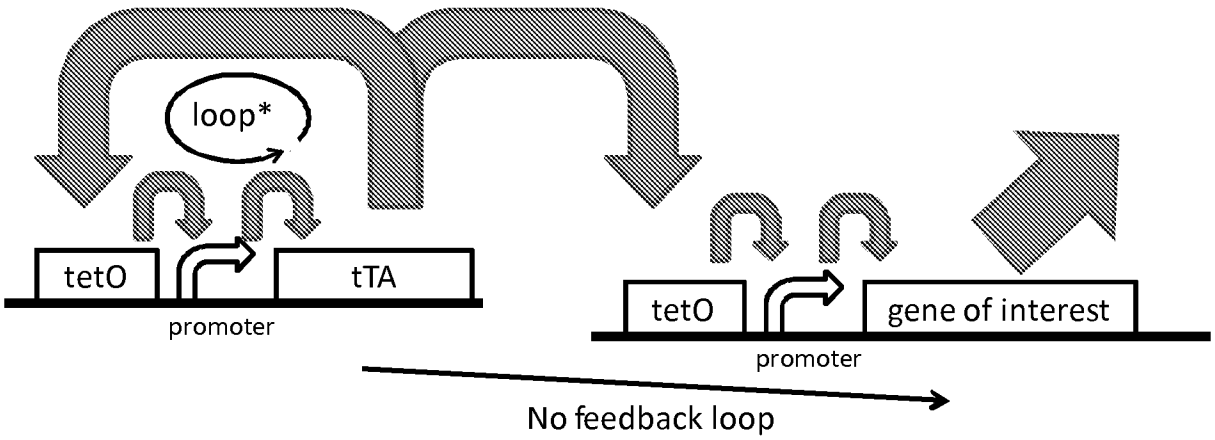
Second element:

[tetO] [suitable promoter] - [gene of interest]

These 'suitable promoters' are *typically* minimal promoters. Minimal promoters, as mentioned in the specification, are promoter elements which can respond to an

enhancer (for instance tetO with tTA bound to it), but drive little expression on their own (or with tetO adjacent but no tTA). This is the simplest case to consider.

The two elements of the invention work together in the following way:



This is analogous to Figure 2 of the present application, but presented as two elements, according to line 21 of page 11 of the specification ("Alternatively,….). The key part here is the positive feedback loop shown by the arrow marked by a star (*). This feedback is missing from Heinrich. Heinrich has two constructs.

In Heinrich, the first construct has:
[Yp1enhancer] – [hsp70 minimal promoter] – [tTA]

The second construct in Heinrich has:
[tetO] - [hsp70 minimal promoter] - [hid]

This is clear from the Methods section and is helpfully illustrated diagrammatically in their Figure 1 (page 8230), reproduced below.

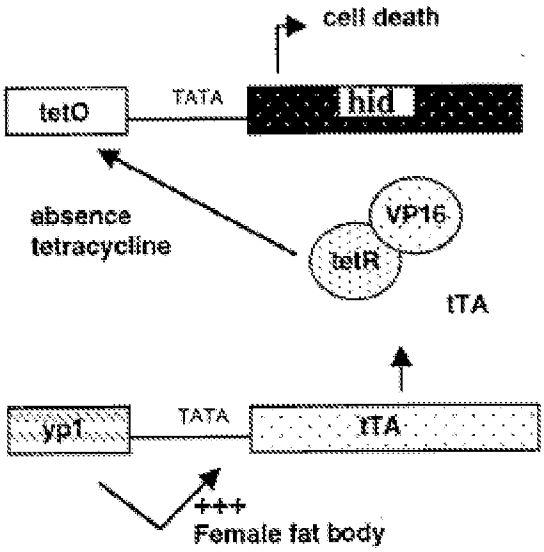


Fig. 1. The tetracycline-regulated female-killing system. Expression of tTA is controlled with the female- and fat-body-specific transcription enhancer from the *yp1* gene (18). In the absence of tetracycline, tTA binds to tetO and induces expression of the proapoptotic gene *hid*. The loss of fat body results in female-specific lethality. In the presence of tetracycline, females are fully viable, because the binding of tTA to tetO is inhibited, switching off *hid* expression.

This is the classic configuration of the tet-off expression system, with no hint of positive feedback. It is apparent that in Heinrich's first construct, the expression of tTA is driven by the **Yp1 enhancer** (acting on an hsp70 minimal promoter). However, it is clear in the reference that there is nothing in Heinrich's first construct that is responsive to tTA.

The tetO enhancer is responsive to tTA, <u>but this is only found in the second element</u> of Heinrich. The Yp1 enhancer of the first of Heinrich's constructs is certainly not responsive to tTA. Therefore, requirement (i) of Applicants' claim is not disclosed in Heinrich. In other words, there is no positive feedback in Heinrich's first element as the

expression product of the control factor gene only acts to drive transcription from the second element and not from both the first and second elements.

Applicants respectfully emphasize that tTA does not act to drive expression from Heinrich's first element. Thus, Heinrich fails to disclose the necessary positive feedback (of tTA on its own expression) according to the present claimed invention. As explained above, in Heinrich, tTA does not drive expression of both, but of only one element (the second element). A further point of distinction over Heinrich is that Heinrich does not teach "on the same construct". All of Heinrich's constructs have either Yp1-tTA **or** tetO-hid, but not both.

In the interest of advancing prosecution and without acquiescing to the rejection, Applicants have amended claim 1 to specify a **repressible** two component system with a positive control factor which controls expression of both components. By contrast, the two components of the Heinrich system are separately controlled – tTA by the *yp1* genetic sequences and the *hid* coding sequence is expressed on the regulatory control of tetracycline responsive genetic sequences (see Figure 1, for example). Heinrich provides a very different solution to the problem of insect control than does present Applicant.

Gossen is characterized as teaching, at the time of the present invention, that tTA and rtTA induce unwanted pleiotropic effects by squelching that may kill a cell, and that the concentration of the tetracycline-controlled transactivator should not exceed a certain intracellular concentration in cell cultures or in transgenic animals. Gossen is said to suggest overcoming the squelching via the creation of autoregulatory loops where the transactivator not only controls expression of a gene of interest but also its own synthesis, i.e. the transactivator gene is under control of the *tet* promoter.

The Patent Office has acknowledged novelty over Heinrich, but Applicant respectfully submits that further clarification is required in view of the Examiner's

comments in the middle of page 4 of the Office Action, in particular. The key point, stressed before, is that nowhere does Heinrich ever indicate a positive feedback system as part of a repressible system, for example, one suitable for control of insect populations. Thus, Heinrich does not teach or suggest that tTA acts on the *yp1* enhancer or *hsp70* promoter (which would have to be present on the first element to correspond to the present claims). In Heinrich, tTA does bind tetO, but here tetO is in the second element. In other words, *yp1* may drive tTA expression, but tTA does not drive its own expression by acting on yp1 (in contrast to the Examiner's comments regarding claim 6 on page 4).

With respect to the cited Gossen reference, please consider the following comments. "Squelching" is known, as acknowledged in the present specification, for example in the overproduction of tTA. Gossen does mention "bi-directional promoters," but note that the present claims require two separate promoters, one per element.

Applicant respectfully notes that present Applicant's 2 promoters might be the same promoter, but if that is the case, then there are 2 copies (1 per element). Thus, the promoters are not shared in the way envisaged in Gossen. The present claimed system may use a bi-directional enhancer, but that is very different structurally than the system of present Applicant.

Again, Gossen lacks any form of positive feedback, as the tTA it discloses is not arranged to act upon its own promoter; instead, Gossen's promoter acts upon both tTA and another coding sequence.

However, the present claims require something different; see the figure provided above. Thus, the relevance of Gossen is queried. Applicant cannot see what it adds to Heinrich, even if they were to be combined. It is not clear that one of ordinary skill in the art would have been motivated to combine the teachings of these references to arrive at the present claimed invention, absent the impermissible use of hindsight.

Applicant provides the following comments concerning the cited Pane reference. This also seems to be only cited as relevant to the codon usage claim, a dependent claim. Pane's teaching is towards the control of alternative splicing of RNA transcripts, focusing on the Cctra intron in *C. capitata*. A system comprising the current 2 elements with a positive feedback loop is completely absent from the disclosure of Pane. Again, Applicant respectfully submits there in no relevance to the present claimed system, other than perhaps to indicate that codon usage is known, which is not disputed in the general state of the art. Applicant does not concede that there is any direct suggestion for combination with the present system. The present claimed invention does not rely on alternative splicing.

As to the cited Fussenegger reference, please consider the following characterization by Applicant. This reference does disclose a positive feedback system, but the structural arrangement of the features therein differs from that of the present claimed invention. The multicistronic approach set out in Fussenegger is to provide a composite minimal promoter and tetO enhancer unit (PhCMV*-1), see the paragraph spanning pages 733 and 734. tTA is one of the cistrons in the Fussenegger construct (i.e., one of the coding sequences), so there is feedback.

However, Fussenegger do not disclose that there are two elements and that each element has its own promoter. There is only one element, and it only has one promoter (the minimal promoter contained within PhCMV*-1. Please see the 4 figures on page 736 of Fussenegger. In the simplest arrangement (top left) : PhCMV*-1 drives expression of GFP, then there is an IRES to allow expression of tTA, followed by the poly A tail. Also provided is *bla* which encodes a beta-lactamase for antibiotic resistance selection, but there is no separate second promoter provided for this: presumably its expression is driven by PhCMV*-1. The remaining three figures are much the same, adding further IRES's and coding sequences to the portion driven by PhCMV*-1. Fussenegger has also provided a different solution to the problem of control of gene expression.

Importantly, no further promoters are provided for driving the expression of these additional coding sequences. In the present claimed system, the second coding sequence, the gene of interest, is provided within the second element and with its own second promoter. Thus, there are significant structural differences and no teaching that any of these should be changed or what they should be changed to.

This reference and the remaining references do not teach or suggest that there is any need for an alternative arrangement and thus teach away from the present claimed system. In fact, Fussenegger is dismissive of any other approach than the one disclosed therein; see the left hand column of page 734, where the "major limitations" and "obvious limitations" of other approaches are mentioned. Thus, Fussenegger reference champions its own multicistron approach at the expense of others. It is clear from the plasmid diagrams on page 736 (plus see lines 3-7 of right-hand column of page 736) what they mean by their approach and, because this is structurally different to what is presently claimed, there is no motivation to change their arrangement. Combining the teachings of this reference, in the absence of the use of hindsight and Applicant's specification and claims, would lead to a different system than that claimed in the present application.

Furthermore, Fussenegger focuses on mammalian systems (see above), whereas the present systems are for controlling insect pest populations, thus introducing a further barrier in the mind of the skilled person to combining any teaching from Fussenegger with any other document.

Indeed, although Gossen is referenced in the first paragraph of Fussenegger's discussion section, the overall context is negative, citing "difficulties" (see line 7) with respect to maintaining tTA levels, so one of ordinary skill in the art would actually be **discouraged** from combining these two documents. Even if combined, it is apparent that significant structural re-organization into two discreet elements, each with its own promoter, is not suggested by or obvious over either reference. Fussenegger would

lead one away from using the teachings of Gossen in creating repressible gene expression constructs of the present invention.

Clearly the art was not settled with respect to how to achieve a regulated gene expression system such as that claimed in the present application, and therefore it is not fair to conclude the present claimed system would have been obvious to create, nor is it fair to conclude that there would have been any reasonable probability of success with the present claimed constructs. Clearly the field of the present invention is very complex, and the appropriate control of gene expression is a difficult goal to achieve.

One of the advantages of the present invention is its application for field use, as mentioned above, because of the loss of function due to loss of linkage during recombination when using separate constructs. Fussenegger seeks to avoid separation or loss of linkage while still driving expression for multiple coding sequences, but to do this, they provide a single promoter and then have to ensure that one (or two or three) IRES's are inserted drive expression of the additional coding sequences.

The present Applicant takes an alternative approach and thereby avoids the strict need for insertion of IRES's, as these are not required in the present two-element system as it provides two promoters instead. The use of IRES's is inherently less preferable for ensuring full expression (compared to a promoter), and can also significantly increase the size of the construct. Instead, Applicant has shown that the expression of the gene of interest from the second element can be driven by an enhancer from the first element, which is **surprising** in itself, see page 11 of the specification. This alternative approach is not hinted at in any of the prior art and is an unexpected advantage.

Applicants have provided an extensive discussion of the cited Heinrich reference above. As Heinrich is completely silent on any form of feedback, it certainly would <u>not</u> have been obvious to introduce this into Heinrich's system by replacing, for instance, the Yp1 enhancer of Heinrich's first construct with one responsive to tTA, to provide the

required "positive transcriptional control factor for (both) (i) the at least one first promoter in said first element" in claim 1.

In the interest of advancing prosecution and without acquiescing to the rejection, Applicants previously amended claim 1 to specific a repressible two component system with a positive control factor which controls expression of both components of the system. This is not taught or suggested by the cited references, alone or in combination; it represents a very different strategy for regulated gene expression than is taught by the cited references, alone or in combination. This key feature does not appear to be found in the cited art. Thus, Applicants respectfully maintain that the invention as currently claimed neither taught nor suggested by the prior art, and it would not have been obvious to one of ordinary skill in the art to construct a system where the same factor controls the expression of both the regulatory factor and the second component of the system.

Moreover, please note that in the present claims, there are embodiments where the lethal gene and the regulatory factor are the same, for example tTA. The cited art does not suggest that this could be so, and with so much information in the field teaching the use of a gene heterologous to the regulatory sequences for lethality or sterility, it is clear that this is not where the art leads one of ordinary skill in the art in seeking a solution for this technical problem. It is by this self-action (autoregulation) that positive feedback in the insects is obtained in the present invention. However, the cited art lack this essential feature. Accordingly, the present inventors have established a new and nonobvious system which can be highly effective in a very wide range of insects. Thus, the present invention has considerable advantages over the prior art.

An advantage of the present invention is that the complete expression system can be introduced with only a single transformation event. This also means that insects homozygous for the system are homozygous at only one locus rather than two, which makes them easier to construct by breeding, and tends to reduce the fitness cost due to insertional mutagenesis.

Accordingly, not only does the present invention provide a promoter with a broad specificity throughout insects, but it also overcomes several problems that tend to occur with expression systems in the field, i.e. in actual insect populations. Thus, the present invention is not obvious over the cited art.

Despite the evident need for such a system, there is no mention of a controllable, positive feedback element in the cited art or any instructions as to how the skilled person may obtain one. Thus, there is nothing in the prior art to motivate the skilled person to provide a system according to the present invention which comprises a positive feedback loop, as neither of these documents suggest why this might be useful, let alone how to provide it. In other words, the cited art, despite disclosing the tetracycline/tTA system, teach completely different pest control approaches to that of the present invention. Note that the for Examination Guidelines Update, published September 1, 2010 in the Federal Register, at 75, 53647, warns that "a proper rejection based on the rationale that the claimed inventions is a combination of prior art elements also includes a finding that results flowing from the combination wo uld have been predictable to a person of ordinary skill in the art," and that "a combination of known prior art elements that would have reasonably been expected to maintain their respective properties or functions after they have been combined." With the problems acknowledged by the cited art, it is Applicant's position that the present claimed invention, with elements put together in particular ways, is sufficiently complex that it would **not** have been predictable from the cited art that the present system could be successful.

In view of the foregoing and the amendments to the claims, Applicants respectfully maintain that the present invention as claimed is not *prima facie* obvious over the cited art, and the withdrawal of the rejection is respectfully requested.

Conclusion

In view of the foregoing, it is submitted that this case is in condition for allowance, and passage to issuance is respectfully requested.

If there are any outstanding issues related to patentability or with respect to this response, the courtesy of a telephone interview is requested, and the Examiner is invited to call to arrange a mutually convenient time.

This response is accompanied by a Petition for Extension of Time (three months) and payment of $555.00 as required by 37 C.F.R. 1.17(a) and $220.00 for the presentation of two independent claims over three as required by 37 C.F.R. 1.17(h). Because 42 claims were pending at the time the filing fees were paid and because the total number of claims upon entry of the present Amendment are 42, it is believed that this amendment does not necessitate the payment of any additional claims fees under 37 C.F.R. 1.16. If the amount submitted or the extension requested is incorrect, however, please charge any deficiency or credit any overpayment to Deposit Account No. 07-1969.

Respectfully submitted,

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